Fowler (G. B.)

THE

SPECTROSCOPE;

ITS

VALUE IN MEDICAL SCIENCE.

BY

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[*REPRINTED FROM THE TRANSACTIONS OF THE NEW YORK ACADEMY OF MEDICINE.*]

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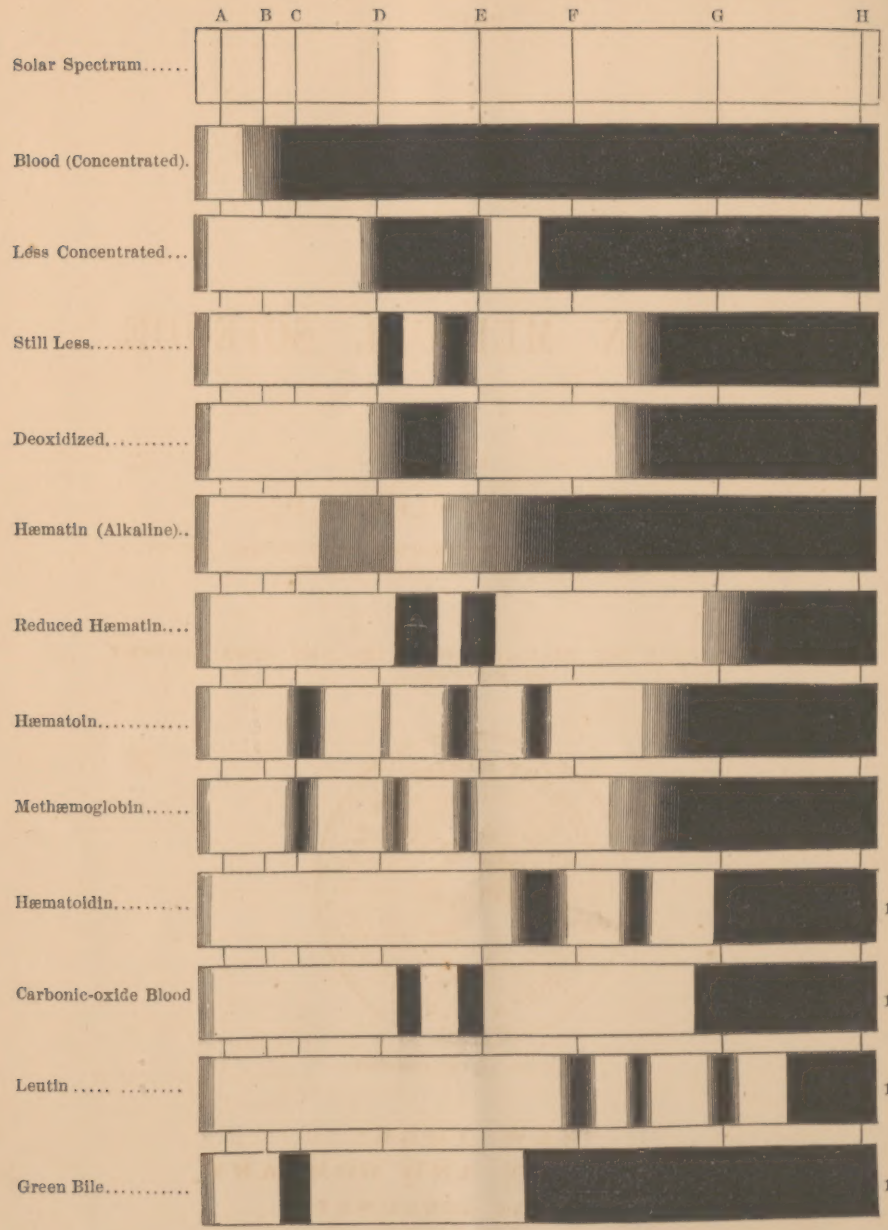

CHART OF SPECTRA.

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THE SPECTROSCOPE, ITS VALUE IN MEDICAL SCIENCE.

By GEORGE B. FOWLER, M. D.,

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Read April 20, 1876.

Mr. President and Fellows of the Academy:

In 1670, or thereabout, Newton discovered the compound nature of white light. That is, he found that when a beam of pure sunlight is allowed to pass through a prism it undergoes two changes. First, the direction is altered, or the rays are refracted; and, secondly, it is no longer white light but broken up into seven distinct colors, each merging imperceptibly into the other, and ranged in the following order: red, orange, yellow, green, blue, indigo, and violet.

According to modern science the reason for this order of colors is found in the theory that luminous bodies impart to the ether waves of different lengths, and while passing through a prism the short waves are most retarded and the long ones least. Now, violet light is composed of short waves and red light of long ones. Therefore the violet is most retarded or bent, while the red pursues almost a straight course through the glass. The red and violet are consequently at each extreme of the spectrum, while the other colors arrange themselves according to their comparative wave lengths.

In 1802 Dr. Wollaston of England announced that the solar spectrum was not a continuous one, but was interrupted by a great many very fine, dark lines. Some years later (1812) a German physicist, Frauenhofer, investigated the character and positions of these remarkable lines and found them to be con-

stant and invariable in location. To the most prominent he applied the first eight letters of the alphabet, *A*, *B*, *C*, etc., and to-day we speak of Frauenhofer line *B* or *E*, or any particular one, to denote the region of the spectrum to which we wish to call attention. (*See* Fig. 1.)

The significance of these lines remained a secret until 1814, when Kirchhoff, in Heidelberg, made the startling announcement that they were due to the absorption of certain rays by the atmosphere surrounding the sun. He showed that if a metal be made incandescent and its light passed through a prism there would appear a number of bright lines in the spectrum, corresponding in position to some of the dark ones of the solar spectrum. Take, for example, the metal sodium or any of its compounds, and, placing a small quantity upon the end of a platinum wire, subject it to the flame of a spirit lamp; immediately the entire flame is yellow with the incandescent sodium vapor. Permit the light from this vapor to pass through a prism, and we will see a very fine, bright yellow line running vertically across the spectrum in the *yellow*.

Having noted the exact position of this line, remove the sodium flame and substitute a beam of sunlight. The beautiful full spectrum now appears, and just exactly where the yellow line of sodium existed is situated the dark Frauenhofer line *D*. Here, then, was a striking coincidence of location, and, regarding the phenomenon as something not to be overlooked, Kirchhoff at first theorized and subsequently proved the dark line at *D* in the solar spectrum and the bright line at *D* in the spectrum of incandescent sodium to be identical, due in each case to the presence of the vapor of the metal sodium. This is the way he arrived at the conclusion: he first saw that in the light of the sun the *D* line was *dark* while the glowing sodium gave a *bright* line. The thought occurred to him that the conditions were reversed, and he proceeded to experiment accordingly; and, without going too much into details, finally established the fact that *when the metal is itself luminous, or the source of light, the lines in*

the spectrum will be bright, but if the source of light be beyond the vapor of the metal the lines will be dark. In other words, if we volatilize sodium in *front* of a bright flame and cause the light from the flame to pass through the vapor before entering the prism the sodium line is *reversed*, it is dark. And so for other metals. Upon these experiments is based the present theory regarding *the constitution of the sun.*[1]

The delicacy of spectrum analysis in detecting metals varies with different ones. Of sodium $\frac{1}{200,000,000}$ of a grain will bring out the bright line at *D*. Of lithium $\frac{1}{60,000,000}$ and of calcium $\frac{1}{1,000,000}$ of a grain is sufficient to produce the characteristic effect.

It has been for some time observed that if the beam of light employed were permitted to pass through a colored fluid, before or after entering the prism, certain marked changes were invariably manifest in the spectrum. *First*, the most refrangible end (violet and blue) is entirely absorbed, and, *secondly*, one or more isolated dark bands of absorption are seen in the other colors, the *same coloring matter giving definite and invariable absorption.*

Very naturally physiologists caught the idea, and soon resorted to spectrum analysis in the cases of the colored animal fluids, blood, bile, and urine.

A brief description of the spectroscope and a few words concerning its technology are in place at this stage.

Spectroscopes are of various patterns and degrees of complication. A convenient form is made by Browning, of London. There is a narrow slit, regulated by a screw, through which the beam of light is admitted to the collimator tube. At the other end of this tube is a convex lens, which collects the rays and sends them parallel to the prism. Here they are

[1] That it is an intense mass of luminous matter in whose composition the metals play certainly an important part, and, being constantly volatilized by the great heat, are present in the atmosphere of the planet, and absorb certain rays according to the law established by Kirchhoff, *that metals in a gaseous state are opaque to those rays which they themselves are capable of emitting when incandescent.*

bent and broken up into the various colors, and are finally collected and magnified in the observing telescope. The observing telescope is arranged in such a manner as to allow of its rotation horizontally around the stand. This movement brings the different parts of the spectrum successively into view. The eye-piece is provided with two very small wires crossed in the centre of the field. These wires are a portion of the measuring apparatus. When the position of a line or band is to be ascertained, the point of decussation of the wires is placed exactly over the object in such a manner that the re-entering angles shall be equally divided by it. Reference is now made to a graduated scale upon the circular top of the stand and to a vernier scale sliding upon it and attached to the observing telescope. The position of the sun lines having been previously determined, it is easy to ascertain the location of absorption lines. Vierordt, a writer upon absorption spectra, proposes a very simple and convenient measuring method.[1] He considers the spaces between the fixed lines, *A*, *B*, *C*, etc., as divided into one hundred equal parts, and then indicates the position of an absorption band by the expression of its distance from any one of them. As *C* 50 *D*. But I fail to see the use of writing "*D*." So, with the exception of writing *the following letter*, this method will be employed in this paper.

I propose to speak of the fluids separately, and in the following order: *Blood*, *Bile*, *Urine*.

[1] *Die Anwendung des Spectralapparates zur Photometrie der Absorptionspectren.* Tübingen, 1873.

There are several other scales in use, but they require an additional tube than is possessed by the simple instrument already described. In this tube there is a scale photographed upon glass and so adjusted and illuminated that it is seen with the spectrum. This extra attachment involves greater expense, and I must say that I have found the method described in the text to be perfectly satisfactory for physiological work.

See "A New Method of Determining the Position of Absorption Bands," etc. By J. C. Dalton, M. D. Trans. New York Academy of Medicine, 1874.

"On a Definite Method of Qualitative Analysis of Animal and Vegetable Coloring Matters." By H. C. Sorby. Philosophical Magazine (4th Series), XXXIV.

Blood.

The coloring matter of the blood has been given a number of different names within the last ten years, which has created considerable confusion, particularly among those who are not especially interested in physiological chemistry, and have not followed the discussions on the subject. We see it called hæmatin, hæmatoin, hematosin, cruorine, hæmatocrystalin, hæmoglobulin and hæmoglobin. Berzelius suggested the term hæmatoglobin. Hemaglobin is employed by Hoppe-Seyler and the Germans, and will be used in this paper. Of some of the others we shall say more further on.

Hæmoglobin has the chemical composition $C_{54\cdot2}H_{7\cdot2}O_{21\cdot6}N_{16}Fe_{\cdot42}S_{\cdot3}$, and is a crystallizable substance. It was first obtained pure by Kölliker[1] in 1849 from the blood of the dog and fishes, and very soon afterward, 1851, by Funke[2] in the blood of man and the horse. The crystals are procured with variable success in different animals. The blood of dogs and guinea-pigs seems to yield them with greater certainty. They possess the same spectroscopic properties as a watery solution of blood, which is much easier to obtain.

Pure defibrinated blood is perfectly opaque in any appreciable volume, and is not suitable for spectroscopic work. But, when a small quantity of water is added, the corpuscles are deprived of their coloring matter, which is perfectly soluble in water, and what is left of the albuminous corpuscle is practically transparent; so that we now have a tolerably pure solution of hæmoglobin more or less translucent according to the degree of dilution.

Suppose we take a cubic centimetre of pure defibrinated ox-blood, and, diluting it with an equal volume of water, place it before the slit of the spectroscope. We shall find everything dark with the exception of a narrow band of bright red. While the eye is still at the instrument add, very gradually, more water, and observe the remarkable change. By degrees

[1] "Microscopical Anatomy." Philadelphia Ed., 1854, p. 714.

[2] "De Sanguine Venæ Lienalis." Lipstadt, 1851.

the red band grows wider, then the *orange* appears. A very slight additional dilution will permit the *green* rays to pass, and now the appearance is that represented in Fig. 3 of the chart. The *red*, *orange*, and a portion of the *yellow* showing at one extremity of the spectrum, and a dark, broad band, with its edges well defined, separated from the darkness of the other extremity by a band of *green* light. The position of this band would be indicated by saying that it commenced at about *D* and extended to about *E* 10. (The position of all absorption bands is more or less affected by concentration of the solution and the thickness of the layer through which the light has to pass.) This, then, is the spectrum of what we should call a pretty concentrated mixture of blood.

Proceeding now to dilute with water, very carefully, another change will take place. Gradually the light appears in the rest of the *green* and the beginning of *blue*. But note the singular operations at work in the broad dark band. It has been divided by a strip of yellowish green and now we have two absorption bands. The first narrow, dark with sharp cut edges situated at the right of *D;* the second broader, its borders blurred and placed just on the left of *E*. *This is the characteristic spectrum of oxygenated hæmoglobin*, that to which Hoppe-Seyler first called attention.[1] (*See* Figure 4, chart.)

It is possible to carry the dilution to a surprising extent before these bands disappear. As water is further added the entire spectrum gradually clears up, interrupted only by the very indistinct and shadowy remains of the two original bands. And it is interesting and important to observe the greater persistence of the first, for when its mate has quite vanished it is still to be seen.

Hoppe-Seyler thought that these phenomena would be of great assistance in ascertaining the nature of the coloring matter and enable him perhaps to follow it through its decompositions to the bile and urine, the color of which fluids have been thought to be due to altered hæmoglobin.

[1] "Virchow's Archiv," xxiii., 1862, p. 446.

He found that alkaline carbonates and caustic ammonia did not affect the spectrum, that is, did not alter the hæmoglobin, but that acids and fixed caustic alkalies decompose it with an accompanying change in the spectrum; but more of this hereafter.

Prof. Stokes,[1] of England, attracted by Hoppe-Seyler's paper, pursued another path of enquiry.

So much having been said about the difference in color between arterial and venous blood being due in the one case to the presence of oxygen and in the other to its absence, he sought the aid of the spectroscope in the matter and performed the following experiments:

Taking a solution of ox-blood which showed the two bands, (Fig. 4) he desired to find out whether they were in any way due to the presence or absence of oxygen. He therefore added reducing agents which would appropriate any free oxygen in the solution. Ferrous sulphate, to which a little tartaric acid had been added and then made alkaline with ammonia, was generally employed. (The agent was to be used with an alkaline fluid and the tartaric acid is put in to prevent a precipitation of ferrous carbonate.) When a little of such a deoxidizing substance is added to our blood solution the first thing noticed is a darkening of color, recalling the hue of venous blood. But for us the most important change has occurred in the spectrum. Placing the altered blood before the slit of the spectroscope we are confronted with a remarkable change. Our pair of distinct bands have disappeared, and a single, dim, broad band is seen commencing at about *D* 10 and extending to *D* 90. The spectrum has also become visible as far as *G*. (Fig. 5, chart.)

Stokes reasoned that this must be the spectrum of reduced blood. The proof was simple. Shake the solution with air and ascertain whether the single band will give place to the original two. It does, and the reduction and oxygenation may be repeated until the color of the reagent begins to interfere with that of the blood. It was ascertained that these

[1] Proceedings Royal Society, vol. xiii., 1864.

changes were not due to the color of the reagent employed, as others perfectly colorless gave the same result.

Stokes also found that, if a full bottle of oxygenated blood solution be tightly stopped and set aside for about twenty-four hours, at the end of that time the color will have changed to that of our reduced hæmoglobin, and the same change in the spectrum will have been effected. Agitation with air will restore the red color and the bands of oxygenated hæmoglobin.

These phenomena are very interesting, and are indeed, as proved by Prof. Stokes, the analogues of what takes place in the blood in the living body. The aërated hæmoglobin represents arterial blood, and reduced hæmoglobin venous.

Stokes's conclusion is "that the coloring matter of the blood is capable of existing in two states of oxidation, distinguishable by a difference in color and a fundamental difference in their action on the spectrum. It may be made to pass from the more or less oxygenated state by suitable reducing agents, and recover its oxygen again from the air."

But it is important to mention here that venous blood as it exists in the circulation does not give the spectrum of venous hæmoglobin. Dr. Sharpey, of London, has demonstrated this fact, and we have repeated his experiments. In order to demonstrate this it is of course necessary to render the blood transparent; but the addition of water is equivalent to aëration. Therefore we must employ water from which all the air has been expelled by boiling in vacuo.

A glass syringe having been filled with this water the nozzle is inserted into a vein and a few drops of blood drawn up. The solution thus contained in the glass cylinder is placed before the spectroscope for examination. And as before stated venous blood under normal conditions gives the two bands of oxygenated hæmoglobin. This is only another of the wise arrangements of Nature where she provides against sudden arrests of respiration proving instantaneously fatal. These experiments prove that there is sufficient oxygen in venous blood to bear another circuit or so and to meet the demands of nutrition. But suppose oxygen is prevented entering the lungs for

a longer time, or is permanently prevented or is displaced from the blood by other gases, what should be the condition of venous and even arterial blood regarding oxidation then? Under such influences it has been found to be completely deoxidized and to correspond spectroscopically with Stokes's reduced hæmoglobin. (Except in case of displacement by some gases, where we have other characteristic spectra.) Of course after the residual air has been exhausted from the lungs the arterial blood will not differ from the venous. In fact all of these phenomena may be studied upon the living animal with the circulating blood. We have simply to employ a transparent living membrane, such as the web of the frog's foot, in the place of our blood solution. Then, although the picture is somewhat indistinct and mottled, on account of the interruption of the light by the tissues, the hæmoglobin bands are easily discerned, and, what is still more important, give place to the single reduced band if respiration is arrested.

A. Schmidt[1] has found that destruction of the medulla oblongata and death by blows upon the head will enable one to see the broad band of reduced hæmoglobin in the blood of the animal. These are only modes of suspending respiration while the circulation is still maintained.

It is evident, then, that the blood in passing through the lungs is oxygenized and afterwards loses this oxygen. How and where does this latter change take place?

We have already seen that it will occur in a solution of blood which is simply allowed to remain at rest protected from the air, and Schmidt, in the course of experiments just alluded to, with a view of determining the reducing power of different tissues upon blood, gives the following results:

If he exposed a muscle of a frog in blood outside of the body and produced continuous tetanus, the oxygen was disassociated quicker than by muscle at rest, but not so rapidly as by a dead muscle. Brain and liver substance reduced a watery solution very rapidly, the solution being at 0° C. The phenomena presented by the full bottle show that the blood

[1] "Centralblatt," 146.

itself contains substances, the nutritious matters, which have a great affinity for oxygen but are not capable of appropriating it so rapidly as the coloring matter, and must needs be in longer contact with it in order to be oxidized. And the experiments of Schmidt demonstrate that the tissues are capable of producing the same change. In the hæmoglobin oxygen is in a state of *physical admixture*, in the other ingredients of the blood and in the tissues it is *chemically combined*.

That oxygen is very loosely combined with hæmoglobin is shown by the displacing power of various gases upon oxygenated blood, and by the results of submitting it to the air pump.[1]

Spectroscopic Effects of Gases and Reagents upon Blood.

Carbonic Oxide, CO.—When a stream of this gas is passed through aërated blood the oxygen is displaced and the carbonic oxide unites with the coloring matter in equal volume.

Before the spectroscope carbonic oxide blood shows very interesting peculiarities. We have two about equally wide and dark bands situated in the yellow between *D* and *E*. To the unpractised eye they might seem identical with the bands of oxy-hæmoglobin. But these latter do not bear so close a resemblance to each other as those now under consideration, and are situated farther toward the blue.

The distinguishing feature of the carbonic oxide bands, however, is to be found in their behavior with both reducing and oxidizing agents.

Neither of these affect them. But, if the solution be allowed to stand for several days at an ordinary temperature, these bands disappear and the broad band of reduced hæmoglobin is substituted.

Hoppe-Seyler first showed that blood in combination with carbonic oxide would not be affected by reducing agents, and Dr. Gamgee[2] found that carbonic oxide would produce the

[1] It has been found that, when blood is subjected to the air-pump, its coloring matter will be deprived of its oxygen when the pressure falls to about twenty-five millimetres of mercury.

[2] "Medical Times and Gazette," 1866, p. 325.

same bands after reduction had been accomplished and even in presence of an excess of the reducing agent. He further pointed out that poisoning by carbonic oxide or charcoal fumes invariably produces carbonic oxide blood.

Nitrogen Monoxide, N_2O (Laughing-gas).—The effect of this gas upon blood is to produce an intense red color. There is, however, no change in the spectrum, and reducing agents will cause the broad band of Stokes to appear.

Carbonic Acid Gas, CO_2, simply displaces the oxygen and shows the spectrum of reduced blood.

Poisons.

Preyer has experimented upon blood with a great variety of reagents, with most of which he obtained characteristic spectra. It is only necessary to mention a few to show that we have a valuable aid in the spectroscope in detecting these agents. (Harley[1] also, it may be mentioned, has published a detailed account of researches in this direction, but with no particular reference to the spectroscope.)

Nitrites.—We are indebted to Gamgee[2] for very interesting reports of the action of this class of substances upon the blood. He found that "all nitrites except that of silver show some crystalline form, color, and spectrum." The blood assumes a chocolate hue under their influence, the two oxy-hæmoglobin bands becoming very faint and another appearing at *C*. Ammonia causes the two bands to grow more distinct, and two entirely new ones (making three) to show themselves in the orange, the blood at the same time resuming its red color.

Oxidizing and reducing agents act perfectly with nitrite-blood. The reducing agent first restores the normal oxy-hæmoglobin spectrum and then induces that of deoxidized; seeming to show that the nitrate superoxygenated the blood.

Nitrites so affect blood as to enable it to resist the action of carbonic oxide. But the loose oxygen is not destroyed or dis-

[1] "Proceedings of the Royal Society," vol. xiii., p. 157.

[2] "Philosophical Transactions," 1868, p. 589.

pelled; it appears to be "simply affected in an unknown manner." Gamgee founds this statement upon the behavior of nitrite-blood with reducing agents; as we have just seen, they first restore the spectrum of oxy-hæmoglobin before inducing the reduction, without the aid of atmospheric air.

Quantitative Spectroscopic Analysis of Hæmoglobin.—We have seen that blood gradually diluted before the slit of the spectroscope permits first the red and next the green light to pass (Figs. 2 and 3). It has been found by Preyer,[1] to whom we owe so much in this department of physiological chemistry, that the degree of dilution which allows the green to show itself is so constant that a sufficiently accurate method of quantitative analysis of hæmoglobin can be based upon it. The process is as follows:

A concentrated solution of a known weight of hæmoglobin crystals is placed in a glass chamber with parallel sides which are one centimetre apart. This chamber or "hæmatinometer" is now placed before the slit of the instrument, and the light allowed to pass through. Then proceed very carefully to add distilled water from a finely graduated burette, and stop the moment green light begins to show. Observe the width of the slit and the position of the light. The solution now contains a percentage of hæmoglobin, which will always be the same under the same conditions. Let K represent this percentage.

Now, if we wish to estimate the percentage of coloring matter in a specimen of blood, we have only to repeat the above process. First, be sure that the conditions as to the light and width of slit are the same. Then take a measured quantity of the blood, agitate it well with air, and dilute it with a small measured volume of water. This blood solution is then placed in the "hæmatinometer" before the slit and further diluted with water from the graduated burette until the green begins to come through. Now we have arrived at the point reached in the first experiment. A little mathematical calculation only remains to ascertain the result. k = the

[1] "Die Blutkrystalle," Jena, 1871, p. 121.

percentage of hæmoglobin present under the given conditions. Let the total quantity of water added $= w$, and the amount of blood employed $= b$. Then we shall have the equation:

$$x = k^{\frac{w+b}{b}}$$

Decomposition Products of Hæmoglobin.

The coloring matter of the blood is subject to spontaneous decomposition, in the body and out of it. These decomposition products can also be produced by treating hæmoglobin or blood with certain reagents.

Those best known and which give definite spectra are: 1, Hæmatin; 2, Hæmatoin; 3, Methæmoglobin; and 4, Hæmatoidin. We shall study them in the order enumerated.

Hæmatin.—$C_{68}H_{102}O_{15}N_{12}Fe_3$.

Caustic Potassa.—If a solution of hæmoglobin or blood be treated with caustic potassa the color becomes brownish and in its spectrum we have a dim broad absorption band commencing at *C* 50 and extending to *D* 40 (*see* Fig. 6, chart).

This is hæmatin. It is an important fact that this substance is capable of oxidation and reduction. The spectrum just described is that of oxygenated hæmatin. Add a small quantity of the reducing agent, and, once more examining the spectrum, observe what has taken place. The shadowy broad band has gone and the *green* and *blue* rays are much less obstructed. The most striking change, however, is the appearance of two entirely new bands, the first situated at *D* 30, and the second on the left of *E*. Both are very black and remind us at once of the bands of oxygenated hæmoglobin. But a little attention will show that they are placed further to the right, and another point of distinction is that the first band of reduced hæmatin is the broadest and appears distinct before the second has begun to show itself (Fig. 7, chart). By agitating the reduced solution with atmospheric air these bands will disappear and the single one of oxy-hæmatin will return. Hæmatin produced by this means was called by Stokes "alkaline hæmatin" to distinguish it from the products by acids.

But it has been shown by Preyer that the "acid hæmatin" of Stokes is not hæmatin, but an entirely new substance which he calls *Hæmatoin*.

Caustic Ammonia.—Hoppe-Seyler describes hæmoglobin as unaffected by this reagent, but we have found it capable of producing hæmatin. It is true that the spectrum is not very decided in the oxygenated state, but reducing agents render the proof. We have found that a solution of blood treated with caustic ammonia placed before the slit of the spectroscope will not give any well-marked band, but simply absorption of the refrangible end of the spectrum up to *E*. Treated with a reducing solution the two black, sharp-cut bands of reduced hæmatin immediately appear, and what is important they can be banished by agitation and made to reappear at pleasure by again reducing them.

Alkaline Carbonates.—Regarding the action of these substances we must again differ from Hoppe-Seyler, and agree with Burdon Sanderson[1] and Stokes that they determine the production of hæmatin. Their action is not so rapid, requiring a day or two, nor is the first spectrum like that of either of the preceding. The reduced spectrum is, however, the same.

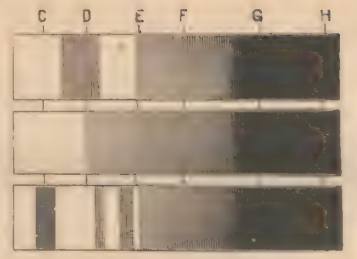
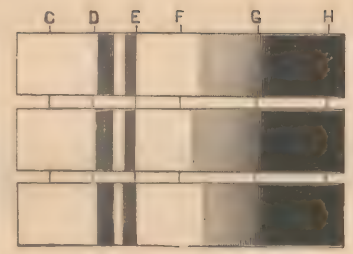
We treated a solution of sheep's blood with carbonate of soda, and examined it after four days, kept at ordinary temperature. It then gave the bands of oxy-hæmoglobin quite indistinctly and another well marked in the orange on the right of *C*. On the application of deoxodizing agents all of these vanished, and the unmistakable pair of reduced hæmatin once more came boldly in view.

I was not able to restore the band near *C* by agitation with air. Those of oxy-hæmoglobin, however, returned more distinct than before. It must have been that the transformation into hæmatin was not complete and the subsequent manipulation so affected it as to render its spectrum invisible.

This completes the description of the methods which have produced hæmatin in our experiments.

[1] "Hand-book for the Physiological Laboratory." American edition, p. 197.

SOLUTION OF BLOOD.

REAGENT.	OXYGENATED.	REDUCED.	REMARKS.
Caus. Potass.....			Can be alternated.
" Ammo.....			" "
Alk. Carb.....			Cannot be alternated.

HÆMATIN.

Other reagents are mentioned by authors as determining the formation of this substance. Stokes gives in addition, acetic and tartaric acids, alcohol, and heat. Dr. Waterman[1] mentions chloroform. They do not claim for these the same spectroscopic features, but say that the position and number of bands depend upon the agent used.

Thus by acetic acid Stokes describes "four banded acid hæmatin," and by a caustic alkali one band "alkaline hæmatin." Now, the "acid hæmatin" was not affected by reducing agents, but, being a product of decomposition of the coloring matter of the blood, he called it hæmatin. We have classed it under Hæmatoin.

Therefore, instead of calling the results of the action of all the above agents *hæmatin*, it appears a better classification to recognize as this substance only those which by deoxidation show the *two bands of reduced hæmatin*, without special regard to their spectral agreement in the oxidized state where, according to my observations, they differ with each reagent. The following table will perhaps be useful. It illustrates the three varieties described in this paper.

It will be seen, from the foregoing table, that the spectrum of hæmatin formed from an alkaline carbonate is identical with the spectrum of methæmoglobin (Fig. 7, chart). But there is no danger of confounding them when we treat each with a reducing agent. The methæmoglobin is unaffected, while the other undergoes the change already spoken of.

Hæmatoin.—When either acetic or tartaric acid is added to a solution of blood, or its crystals, in large quantity, the formation of hæmatoin (Preyer), acid hæmatin (Stokes), iron free hæmatin (Hoppe-Seyler), is determined.

The most certain method of obtaining the spectrum of this substance is that advised by Stokes. Take a tolerably concentrated blood solution and to it add equal volumes of acetic acid and ether. Mix well but not violently, for an emulsion will form. Allow the ethereal extract to collect at the top, and, having carefully poured it off, subject it to spectroscopic

[1] *Medical Record*, New York, October 15, 1874, p. 532.

examination. There will four bands appear (Fig. 8, chart), one broad and plain at *C*, one narrow and very dim at *D*, a broad and well-marked one at *D* 75, and the last, broad, with shadowy outlines, at *E* 70.

We have not always succeeded in seeing the *D* band, and in order to do so the solutions must be varied regarding concentration, and the light delicately regulated at the slit.

I think the effect of tartaric acid alone is sometimes as satisfactory as the ether solution, and would especially recommend it to reveal the band at *D*.

Hæmatoin is not capable, like the foregoing substances, of existing in two states of oxidation; reducing agents do not affect it, nor does agitation with air.

Methæmoglobin.—This is a substance found in old extravasations, hydrocele and ovarian fluids, and is a product of the decomposition of hæmoglobin. It was first described by Hoppe-Seyler and named by him. The composition of this substance is not very well understood. It can be prepared in several ways. If a dilute solution of blood is kept for several days at ordinary temperature, or if *very small quantities* of any acid are added to a more concentrated specimen, methæmoglobin will form. These methods amount to the same thing in the end, for in stale blood there are acids developed, formic and butyric, which give a distinct reaction to test paper.

Examined with the spectroscope it shows, in addition to the characteristic blood bands (Fig. 4, chart), another dark band at *C*. Treated with reducing agents these all disappear with the production of the single band of reduced hæmoglobin (Fig. 5, chart). This is because the methæmoglobin band *C* depends upon an acid condition, and our reducing agent being alkaline destroys it, and sufficient hæmoglobin is present to respond in its well-known manner.

Give the hæmoglobin time to decompose and we shall have pure methæmoglobin, which, according to Preyer,[1] gives a spectrum similar to hæmatoin. It would seem, then, that methæmoglobin is simply partially-formed hæmatoin.

[1] "Die Blutkrystalle," Jena, 1871, p. 191.

Considerable care is necessary in producing this substance with acetic and tartaric acids. A few drops of a dilute solution are sufficient to bring the *C* band into view. If a greater quantity is added the blood bands gradually fade, the spectrum at the same time clearing up in *green*, *blue*, and *violet*, and shortly, as in the case where hæmoglobin is allowed to entirely decompose, the four bands of hæmatoin are given. A concentrated solution of blood should be taken to begin with, and never add enough of the reagent to destroy the hæmoglobin bands.

In preparing methæmoglobin by adding acids, the position of the absorption in the orange varies according to their strength. The stronger the acid the farther toward *B* will the band be situated. By using stale blood and adding an excess of acetic acid the band near *C* does not shift, but contracts upon both sides (Preyer). We have found nitric and sulphuric acids to be equally capable of giving the spectroscopic characters of methæmoglobin.

Hæmatoidin.—This is another of the decomposition products of the normal blood color first described by Everard Home in 1830, and subsequently brought to light again by Virchow[1] in 1847. It is crystallizable and its crystals are easily discovered in extravasated blood of the corpora lutea, apoplexy, sputa of pneumonia, and in obliterated veins.

Hoppe-Seyler[2] states that this substance is identical with *bilirubin*. But Preyer[3] asserts that they are distinct, in that their spectra are unlike. This difference can be seen when a chloroform extract of gall-stones is made. We then have a bright yellow fluid, which before the spectroscope gives no well defined absorption bands either in dilute or concentrated solution (bilirubin). On the other hand, a chloroform solution of an apoplectic clot or corpora lutea of the cow gives us a yellow fluid, which exhibits distinct and characteristic bands

[1] "Archiv für pathologische Anatomie," u. s. w., Band I., § 383.

[2] "Handbuch der Physiologisch- und Pathologisch-chemischen Analyse," Berlin, 1870, p. 178.

[3] "Die Blutkrystalle," Jena, 1871.

(Fig. 10, chart). Very intense illumination is necessary to show this spectrum.

Lectine.—This is the yellow coloring principle which is generally said to exist in the yellow bodies of the ovary, in the yolk of eggs, and, according to Thudichum, in certain vegetables.

It is soluble in alcohol, chloroform, and ether, and its solution presents a decidedly yellow color.

Some confusion exists as to the indentity of lentine, bilirubin, and hæmatoidin. A chloroform extract of the cow's ovary we have seen gives the spectrum of hæmatoidin. We have failed to obtain any satisfactory results in the examination of ovary extracts and fluids from ovarian tumors. Preyer took an ovary in which hæmatoidin crystals were very abundant, and made a chloroform extract of it. Before the spectroscope only the bands of hæmatoidin were seen. But a similar extract of the yolk of egg gave an entirely different spectrum; so this observer holds that the corpora lutea contain no lentine, but hæmatoidin. As regards bilirubin we have seen that it gives no definite bands, and therefore there is no danger of confounding it with either of the above. The characteristic spectrum of lentine is furnished by the extract of the yolk of egg (Fig. 12).

Myochrome.—This is the coloring matter of red muscular tissue, and can be extracted by maceration in water. We shall then have a yellowish fluid, which before the spectroscope presents all the characters of hæmoglobin; carbonic oxide and other reagents affecting both alike.

Bile.

The color of the bile varies in different animals, and sometimes in the same animal, from a green to a reddish brown. This great diversity is due to the presence of two entirely distinct coloring matters, *bilirubin* and *biliverdin*. The intermediate tints would naturally depend upon the relative increase of either of these pigments and produce shades of green and red accordingly. It is on this account that ox bile

may be perfectly green, olive, olive-brown and reddish-brown. Other animals do not furnish quite so much variety in color. Human bile is more or less of a golden-brown, sheep's bile is green, pigs' bile is reddish-brown, rabbits' bile olive-green, dogs' bile golden-bronze, etc. It was at one time supposed that biliverdin and the green coloring matters of plants were identical, but Prof. Stokes [1] proved by spectrum analysis that they are not.

It is a favorite and fascinating idea among some physiologists that these bile-coloring matters are derived from the blood; that they are products of the decomposition of hæmoglobin. Experiments on this subject disagree in a remarkable manner.

The most recent and elaborate were performed by Steiner, in Berlin, and Tarchanoff, of St. Petersburg, who worked in Hoppe-Seyler's laboratory.

The object of the experiments was to determine whether, by injecting water and solutions of blood into the circulation, bile pigment would show itself in the urine. Injections of water were intended to dissolve the corpuscles and set free the coloring matter; blood solutions to supply an excess of the same; in either case to furnish favorable conditions for transformation.

Steiner, in twelve cases with rabbits, injected 10 c. c. of water at 100° Fahr. into the carotid arteries—two of these showed bile color in the urine. In an equal number of injections into the veins no bile color appeared in the urine, nor did the blood color come through the kidneys. The result of six experiments with 20 c. c. of water was negative. But, when from 30–50 c. c. were injected, bloody urine resulted, and *post mortem* examination revealed bloody serum in all the cavities, *but no bile color*.

Tarchanoff thinks that Steiner did not take sufficient precautions, and publishes the result of his own investigations.[2] He used dogs, considering the slimy urine of rabbits not suit-

[1] "Proceedings Royal Society," 1864, xiii., p. 144.

[2] "Archiv für Anatomie und Physiologie," 1873, ii., p. 160.

able. He also guarded very carefully against foreign matter by inserting canulæ directly into the ureters, from which to collect the urine.

The animals were freely fed with meat before the operation. A pure solution of hæmoglobin was prepared and injected into the circulation, followed by very evident bile color in the urine. Injected 150 c. c. of water with a similar result. Steiner, he thinks, must have therefore overlooked these results in his own experiments, or what is very probable hares and dogs give different results.

This, then, is a glimpse at the literature of the subject.

It was thought that the spectroscope might throw some light upon the question. But unfortunately those who thus examined the bile said that it gave no definite spectrum.

A paper on the "Spectrum of Bile," by Prof. Dalton,[1] in 1874, however, gives a new impetus to the subject; for he has discovered that there is a reliable character in the bile spectrum, which we ourselves, having repeated his experiments, can testify to and shall proceed to describe.

Prof. Dalton first describes the visible spectrum of all kinds of bile as being very short and terminating very suddenly at or about *E* 45. He next states that he has found a dark, well-marked band at *C*. He says: "According to my own observation it is so constant and so well marked as to form a characteristic feature in the spectrum of bile whenever it has a decided greenish tint, and often when it is of a yellowish, reddish, or olive-brown color. In eleven specimens of ox bile, sheep's bile, and dogs' bile, of a green, greenish-olive, olive, or olive-brown color, this band was visible when examined in a thickness of only two or three centimetres. In all the nineteen specimens of ox and sheep's bile which had a greenish or olive tint, when viewed in a thickness of two or three centimetres, the band at *C* was very distinct and often quite dark or almost black. In the three remaining specimens which were of a yellowish-brown or olive-brown color, it was distinct and sometimes very dark in layers of two centimetres;

[1] *New York Medical Journal*, June, 1874.

and in a sixth it was perceptible in layers of three centimetres."

We have examined nine specimens of ox bile, six of sheep, one of dogs, and one of rabbits.

A detailed account is as follows:

Ox bile.—Specimen No. 1. Brownish-red. Removed from the body one hour. Viewed in thickness of from 2–5 centimetres, showed indistinct band at *C*. Spectrum ends at *E*.

Specimen No. 2. Olive-green. One hour removed from body. Thickness of from 2–3 centimetres, very distinct band at *C*.

Specimen No. 3. Dark green. One hour after removal. Thickness of centimetre reveals exceedingly dark band at *C*. Spectrum stops at *E*.

Specimen No. 4. Dark green. One and a half hour after removal. Two centimetres thick gives well-marked band at *C*. Spectrum terminates at *E* 50. This specimen is so transparent that the most satisfactory thickness to employ is five centimetres, when the *C* band is absolutely black and sharp cut at its edges.

Specimen No. 5. Olive-green. One and three-quarters hour after removal. *C* band visible in layers of from 1–3 centimetres.

Specimen No. 6. Olive-brown. One hour after removal. Shows no sign of the band at *C*. Spectrum ends at *E*.

Specimen No. 7. Olive-green. One hour after removal. In thickness of five centimetres. The *C* band is distinct. Spectrum ends at *E*.

Specimen No. 8. Olive-green. One hour after removal. In layer of five centimetres, shows *C* band very plain. Spectrum ends at *E* 30.

Specimen No. 9. Olive-green. One hour after removal. In layer of four centimetres, shows band at *C* very distinctly.

Sheep's bile.—The six specimens were all similar in color (dark green) and were removed from the body one hour before

examination. In thickness of from 1½–3 centimetres the band at *C* was in every case very distinct.

Dog's bile.—Golden-bronze color. Forty minutes after removal. Layer of one centimetre showed *C* band very dark and well marked at its edges.

Rabbit's bile.—Dark green with slight olive tint. Four hours after removal from the body. Layer of one centimetre reveals *C* band. These results added to those of Prof. Dalton cause us to wonder at the negative results arrived at by previous observers.

The only specimens in which the *C* band seems to be ever absent are those which have a more or less brown or reddish tint. Never in green bile have I failed to see this band (*see* Fig. 12, chart.) and it appears from those facts that the band at *C* is characteristic of the green element in bile. This is shown experimentally as follows: Add nitric acid or tincture of iodine to a specimen of yellow bile which does not show the *C* band. (These agents oxidize the solution with the effect of rendering the color green.) This specimen will now give the *C* band. The green color is due to oxidation, and the power of oxidizing substances to strike a green color with bile has been long known; but Dalton has found that if a full and tightly-stoppered bottle of green bile, which shows the band at *C*, be set aside for twenty-four hours, the green color will fade and give place to an olive or brown, and will then fail to reveal the *C* band; subsequent exposure to the air or addition of oxidizing agents will restore the green color and its absorption band. This behavior is analogous to that of blood when treated in the same way.

Bile also shows several other bands, but which are not constant or well defined in every instance. These bands seem to have nothing in common with the *C* band. That is they appear sometimes in green and sometimes in brown bile. The most constant are a faint, broad one on the left and a dark, narrow one on the right of *D*.

Does the fact that bile possesses a characteristic spectrum, then, throw any light upon the question as to the origin of its

pigment? Are we able to trace it to any of the products of hæmoglobin? As yet we must answer in the negative—we must await the result of investigations based upon Prof. Dalton's discovery.

Pettenkofer's Test for Biliary Salts.

As is well known, this is a color test. It depends upon the solution changing to a fine purple. But it has been found that there are other substances which, by treating with cane sugar and sulphuric acid, will present the same color. Such are various albuminous bodies, the salts of opium, theine, ethereal oil, cerebric acid (Thudichum), amyl alcohol, etc. But the spectroscope will decide whether the colored reaction be due to either of these or to the bile salts.

The purple reaction characteristic of Pettenkofer's test is usually too concentrated even in very thin layers for spectroscopic analysis. Water, added with a view to dilute it, only renders it still more opaque by precipitating cholic acid. But it has been found by Prof. Dalton[1] that, if a very dilute watery solution of the sodium glycocholate (1–500) be taken to begin with, subsequent dilution with water will not cause a precipitate. Therefore it is well to use very dilute solutions of the suspected substances if they are to be placed before the spectroscope. (Should it be necessary, sulphuric acid may be used to effect the dilution.)

Pettenkofer's test in watery solution shows one broad and distinct band at *E*, the refrangible end of the spectrum being invisible up to *G*. In alcoholic solution the same band at *E* is seen with another not so broad at *F*. Egg albumen treated with Pettenkofer's test shows a broad, shadowy band commencing at about *D* 60, and extending to *F*. The spectra of codeine and morphine are the most like those of the bile-salts, showing a band at *E*; but this band is not near so well defined, and disappears suddenly on the addition of a small quantity of water, while in the case of the latter a considerable dilution is necessary to destroy it.

[1] "Human Physiology," Philadelphia, 1875, p. 214.

Urine.

It was expected that the spectroscope would reveal some positive information as to the origin of the coloring matter of the urine. But it is found that this fluid does not yield any satisfactory results by prismatic analysis. Ordinary urine shows no absorption bands at all, and a specimen which I evaporated from 30 ℥ to 5 ℥, thereby causing it to present a very dark, reddish color, still gave a negative result.

But by adding nitric acid and allowing to stand I have once succeeded in getting a broad, indistinct band at *F*. This band has been seen by other observers, and, because the urine sometimes shows it, there is a theory that urochrome is derived from the bile colors, in that by extracting dog's bile with hydrochloric acid we obtain a substance which gives a very similar band. Further treatment of urine and the bile-extract brings about other spectral changes, which give strength to the theory just stated (Sanderson).

Coming now to inquire into the practical worth of spectroscopic analysis, we are reminded that there is not such a distinction to be made between the two terms "scientific" and "practical" as is generally accepted. The fact is, we never know how very practical a very scientific discovery may at any moment become. It was a scientific course of experiments which preceded the practical use of electricity; the investigations of Goodyear and others into the properties of india-rubber were strictly scientific, and led to the most practical results. And so of almost every useful invention.

Now, physiological spectroscopy is in its infancy, and we may reasonably look for important practical results within the near future. Let us see what it has already accomplished. Stokes's experiments have materially added to our knowledge of the respiratory processes, showing conclusively that the function of the red coloring matter of the blood-corpuscles is to absorb, convey, and deliver oxygen; that the difference in hue between venous and arterial blood is due simply in the

one case to the absence, and in the other to the presence of oxygen.

But it is in the field of forensic medicine that we shall find the spectroscope of absolute use. The detection of blood-stains by the microscope depends upon the presence of the corpuscles. But these elements are very easily dissolved, or otherwise rendered unnatural. Here the microscope would fail; but the spectroscope will reveal reliable signs, as we have seen that even the decomposition products of hæmoglobin give characteristic spectra.

Specimens of blood, wet or dry, kept for long periods undergo changes which render the microscope useless as a means of detection. But, spectroscopically, the presence of blood can be absolutely proved.

For instance, dried blood (on rags, wood, glass, etc.) kept for some days turns brown. It is now practically insoluble in water, and, in order to subject it to examination, we must dissolve in a weak acid. This at once destroys the corpuscles, and the microscope is valueless. But by prismatic analysis we see the two hæmoglobin bands, and in addition one at *C*. It is either methæmoglobin or hæmatoin. Treatment with reducing agents will determine which, and absolutely prove the presence of blood. The broad band of reduced hæmoglobin will appear, if it is the first; if the second, they will have no effect.

Letheby and Sorby examined stains on linen seventeen years old, and the spectra were in every respect characteristic.

We have a specimen of pure sheep's blood which has been kept at ordinary temperature more than a year. The corpuscles have disappeared, it is swarming with bacteria, and the microscope can furnish no proof that it is blood, examined as it stands. Yet it shows the absorption bands of hæmoglobin, and reacts perfectly well with deoxidizing substances. Benoit[1] speaks of a similar observation. Sorby kept dried blood (on rags) exposed to the air, and found that it was converted into methæmoglobin, with different degrees of rapidity, according

[1] "Études spectroscopiques sur le Sang," 1869, p. 60.

to the locality. In towns and in inhabited rooms the change took place sooner than in the country well removed from houses. He also found that dried stains sealed in tubes required three months to change; if sealed wet, they did not change at all. Cited by Letheby.[1]

Some vegetable and animal coloring matters may confuse us at first, but the application of oxidizing and reducing agents will give conclusive evidence of the presence of blood.

Substances most likely to be confounded with blood-color are, an ammoniacal solution of carmine, and solutions of iron sulphocyanide. These, when placed before the spectroscope, show such different absorption as to enable one readily to distinguish them from blood and from each other. An important test for vegetable colors is ammonia. I believe there is not an instance where this reagent does not markedly change the hue, while it has little or no effect upon blood.

Regarding the question, Does pristeatic analysis enable us to distinguish human blood from that of the lower animals? the answer is, It does not. The spectrum is alike in all red blood. (Hoppe-Seyler, Valentine, Bert, Benoit.)

The presence of other coloring matters does not interfere with the detection of blood. I have found that one drop of blood added to an ounce of green ox-bile gives its characteristic absorption very distinctly.

It is sufficient to call attention to what has been said of the action of gases and poisons upon blood to show how important the spectroscope would be in cases of death from any of those substances. With some of them it would be the only means of determining the cause of death, while with others it certainly would greatly strengthen the evidence adduced. The delicacy of this method of examination is very great in the case of blood.

Sorby states that $\frac{1}{100}$ of a grain could be made to show all the transformations, and he says that by a little practice $\frac{1}{500}$ or $\frac{1}{1000}$ of a grain can be detected.

It is further said that with the Sorby-Browning micro-

[1] "Spectrum Analysis." Reports, London Hospitals and Clinics, 1866.

spectroscope a single blood-corpuscle will show the characteristic absorption. Indeed, the first recorded case of the use of the micro-spectroscope in a murder case is where the suspected criminal was near being acquitted on account of the failure to establish the proofs that there were any evidences of blood upon a hatchet which was found in the woods near the victim, and with which, from the character of the wounds, it appeared he must have been attacked. The implement had lain exposed to the weather several weeks, and the most careful examination failed to discover the slightest traces of blood until it occurred to the expert to remove the handle. Then, upon the end which was inserted into the iron ring, a dark, reddish stain was seen. Careful sections of the wood were made, the stain dissolved in a few drops of acidulated water, and, with a $\frac{1}{25}$ inch objective on the micro-spectroscope, this fluid showed the indisputable blood bands, and the prisoner was convicted.[1] Herapath[2] estimates that there was about $\frac{1}{1000}$ grain of blood in the portion examined.

In conclusion, let us say that this is a strikingly beautiful means of investigation: it is free from the objections which chemical analyses of the coloring matters offer, viz., numerous apparatus and reagents. With coloring matters other than blood, I think its chief value, however, at present is in verifying other analytical methods. But we hope by improved illumination and construction to enlarge its field and render its use even more valuable.

I am greatly indebted to my friend Dr. Charles A. Doremus, for his kind assistance in the demonstrations which accompanied this paper; especially for the skillful manner in which he projected the various spectra upon the screen.

[1] "Mountain-Ash Case." Reg. v. Robert Coe, Swansea Spring Assizes, 1866.

[2] Herapath, "On Use of Spectroscope and Micro-Spectroscope in Discovery of Blood-Stains," etc. *Chemical News*, English edition, xvii., No. 431, pp. 113 and 224.